# Verticillium Wilt of Cotton: Epidemiology of *Verticillium dahliae*

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## Introduction

In a cool, wet season, the incidence of Verticillium wilt disease in cotton can cost the Australian industry millions of dollars in lost yield. Until recently, little was known about the isolates of *Verticillium dahliae* prevalent in the cotton production regions of Australia. As a consequence, it has been virtually impossible to study the population diversity, mode of spread and degree of persistence of these pathogens in the field. However, with the advent of molecular biological techniques such as genetic fingerprinting, it is now possible to identify the strain of pathogen in an outbreak of plant disease, to characterise it, and possibly, to isolate genes associated with pathogenicity.

The sensitivity of genetic fingerprinting rests in the ability of the procedure to detect the rare or subtle differences that exist between the genes of one individual and another. In a typical experiment, discrete subsets of the genetic material (DNA) of two or more organisms are analysed for genetic similarities and differences, and calculations are made as to the likely relatedness of the organisms based on the observed genetic similarity. In the present investigation, we have applied the RAPD-PCR technique[1] to strains of *V. dahliae* isolated from cotton plants from a range of production regions in Australia. By using genetic fingerprinting to identify different strains of *V. dahliae*, we hope to achieve a better understanding of the epidemiology of Verticillium wilt disease in cotton.

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[1] RAPD-PCR (Random Amplified Polymorphic DNA - Polymerase Chain Reaction) is a rapid molecular genetic technique which samples random regions of an organism's DNA and produces a pattern of DNA pieces or fragments not unlike a supermarket barcode. Alignment of the 'barcodes' of two or more organisms can reveal both similarities and differences between the individuals and can therefore provide a measure of their genetic similarity. For technical details of the procedures employed in this research, see Ramsay *et al.*, 1996.

**TABLE 1.** List of *Verticillium dahliae* isolates characterised in this survey[2]

| Isolate | Host | Strain | Collection | | | |
|---|---|---|---|---|---|---|
| No. | Variety | Colour | Farm/Grower | Area | State | Date |
| 1001 | Sicala 3-2 | White | Northcote | Boomi | NSW | 03.90 |
| 1002 | Deltapine 90 | White | Benwerrin | Croppa Creek | NSW | 03.90 |
| 1003 | Siokra S324 | White | Drayton | Breeza | NSW | 03.90 |
| 1004 | Deltapine 90 | White | Latoka | Bourke | NSW | 03.90 |
| 1006 | Deltapine 61 | White | Benwerrin | Croppa Creek | NSW | 02.84 |
| 1007 | Deltapine 61 | Black | 20 Stone | NW Warren | NSW | 02.84 |
| 1009 | - | W/B | - | Bourke | NSW | 02.92 |
| 1010 | Sicala 33 | Black | Iffley | Collarenebri | NSW | 03.91 |
| 1014 | Deltapine 61 | White | Benwerrin | Croppa Creek | NSW | 02.84 |
| 1015 | Deltapine 61 | White | 20 Stone | NW Warren | NSW | 02.84 |
| 1016 | CS 189 | White | Kerribee | Merah North | NSW | 03.91 |
| 1018 | Sicala 33 | W/B | Iffley | Collarenebri | NSW | 03.91 |
| 1019 | Deltapine 90 | White | Burratipi | Trangie | NSW | 03.92 |
| 1021 | Siokra L22 | White | Korolea | Boggabilla | NSW | 03.92 |
| 1029 | Sicala V-1 | W/B | T. Porter | Brookstead | Qld | 02.93 |
| 1030 | CS 189 | W/B | L. Brazzel | Brookstead | Qld | 02.93 |
| 1031 | Siokra L22 | W/B | Brownlie | Theodore | Qld | 04.93 |
| 1032 | Siokra 1-4 | W/B | Brownlie | Theodore | Qld | 04.93 |
| 1034 | Siokra L22 | Black | Cooneah | Dalby | Qld | 04.93 |
| 1036-1056 | Sicala V-2 | Black | Auscott 7 | Narrabri | NSW | 03.94 |
| 1057-1069 | Sicala V-2 | Black | Auscott 8 | Narrabri | NSW | 03.94 |
| 1070-1093 | CS 50 | Black | Milawa | Warren | NSW | 03.94 |
| 1094-1099 | CS 189+ | Black | Milawa | Warren | NSW | 03.94 |
| 1100 | cv 84009-47 | Black | Drayton | Breeza | NSW | 03.94 |
| 1101 | Sicala V-1 | Black | Undoolya | Boggabri | NSW | 03.94 |
| 1102 | Deltapine 6100 | Black | Dundee | Cryon | NSW | 03.94 |
| 1103 | - | Black | Ferguson | North Bourke | NSW | 03.94 |
| 1104 | CS 189+ | Black | Hillview | Croppa Creek | NSW | 03.94 |
| 1105 | Sicala 34 | Black | Latoka | Bourke | NSW | 03.94 |
| 1106 | CS 50 | Black | Retreat | Baan Baa | NSW | 03.94 |
| 1107 | Siokra L22 | Black | Northcote | Boomi | NSW | 03.94 |
| 1108 | CS 189+ | Black | Waverly | Burren Junction | NSW | 03.94 |
| 1109 | Siokra L23 | Black | Warilea | Maules Creek | NSW | 03.94 |
| 1110 | CS 189+ | Black | Leopard | St George | Qld. | 03.94 |
| 1111 | Siokra L23 | Black | Leopard | St George | Qld. | 03.94 |
| 1112 | Sicala V-1 | Black | Plantation | St George | Qld. | 03.94 |
| 1113 | CS 50 | Black | Plantation | St George | Qld. | 03.94 |
| 1114 | CS 50 | Black | Bilorey | St George | Qld. | 03.94 |
| 1115 | CS 50 | Black | Snow Farm | St George | Qld. | 03.94 |

[2] Verticillium fungus was isolated from infected cotton plant stems, and cultures were identified using standard morphological and molecular genetic procedures (Ramsay *et al.*, 1996). Variation in colour (white, black or white/black) and growth pattern (fast, slow) was observed among the isolates. This indicated the existence of a complex population structure of *V. dahliae* in these cotton production areas.

**An epidemiological survey of *V. dahliae* in Australian cotton**

With the assistance of cotton pathologists in NSW and Queensland, isolates of *V. dahliae* were collected from the Bourke area [4 isolates], the Darling Downs (Brookstead and Dalby)[3], the Gwydir Valley (Collarenebri and Croppa Creek)[6], the Macintyre Valley (Boggabilla and Boomi)[3], the Macquarie Valley (Trangie and Warren)[3], the Namoi Valley (Baan Baa, Boggabri, Breeza, Burren Junction, Cryon, Maules Creek and Merah North)[8], the St George area [6], and around Theodore [2]. Two fields of cotton near Narrabri [34 isolates] and two near Warren [30] were also targeted for intensive collection of Verticillium wilt infected plants to examine population diversity on a local scale. The host cultivars and collection details of the 99 *V. dahliae* isolates are presented in Table 1.

The genetic fingerprints of all 99 *V. dahliae* isolates were obtained by RAPD-PCR in order to compare their genetic relatedness. This analysis revealed that the isolates could be classified into 15 different RAPD-PCR groups (RGs), with each member of a group showing greater than 80% genetic similarity (greater than 80% of their RAPD-PCR fragments in common) with the other strains in that group (Figure 1). The 15 RGs could be subsequently grouped into 4 major clusters (A, B, C and D) based on a level of 70% genetic similarity.

**Isolates of *V. dahliae* from different cotton production regions**

The largest of the RAPD-PCR groups (RG11), comprised isolates from all eight cotton production regions indicating the widespread nature of this particular genetic type (Figure 2). Representatives of each of the other 14 RGs appeared to be more localised, as they were found in, at most, three different regions. Indeed, members of RGs 2, 3, 4, 6, 7 and 14 were only isolated from a single production region, and so may represent rare genetic types.

Members of RGs 1, 2, 3, 4, 5, 6, 7, 13 and 14, representing almost all of the isolates in the major clusters A, B and D, were only isolated from the

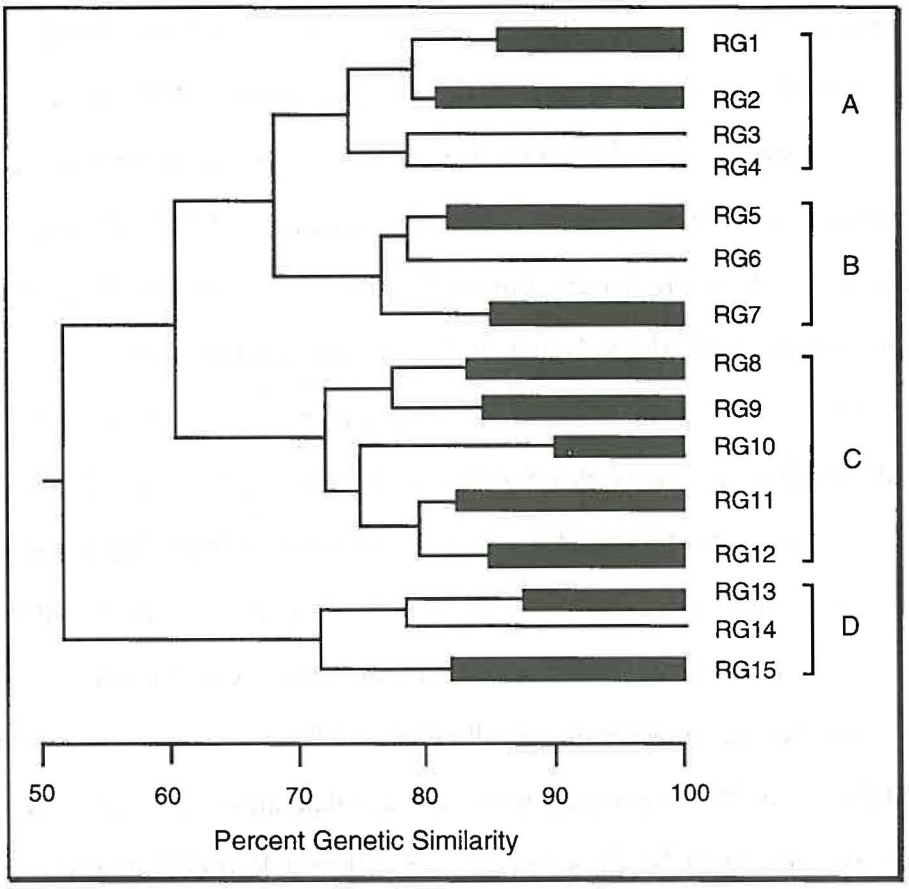


**FIGURE 1.** Tree diagram showing the percentage genetic relatedness of the *V. dahliae* isolates and their classification into 15 RAPD-PCR groups (RGs). The thick black bars signify that two or more closely-related *V. dahliae* isolates belong to the corresponding RG. Four major clusters (A, B, C and D) are also shown.

intensively-sampled fields in the Macquarie and Namoi production regions. Furthermore, isolates from these surveys were also represented in RGs 8, 9, 10, 11 and 15. These findings imply that *V. dahliae* populations are often extremely diverse and that more intensive sampling of fields in the other production regions may also reveal significant strain diversity.

### Isolates of *V. dahliae* from different cotton cultivar hosts

Strains of *V. dahliae* included in this study were isolated from Verticillium wilt-sensitive cotton cultivars such as CS 50, Sicala 33, Siokra S324, Siokra L22

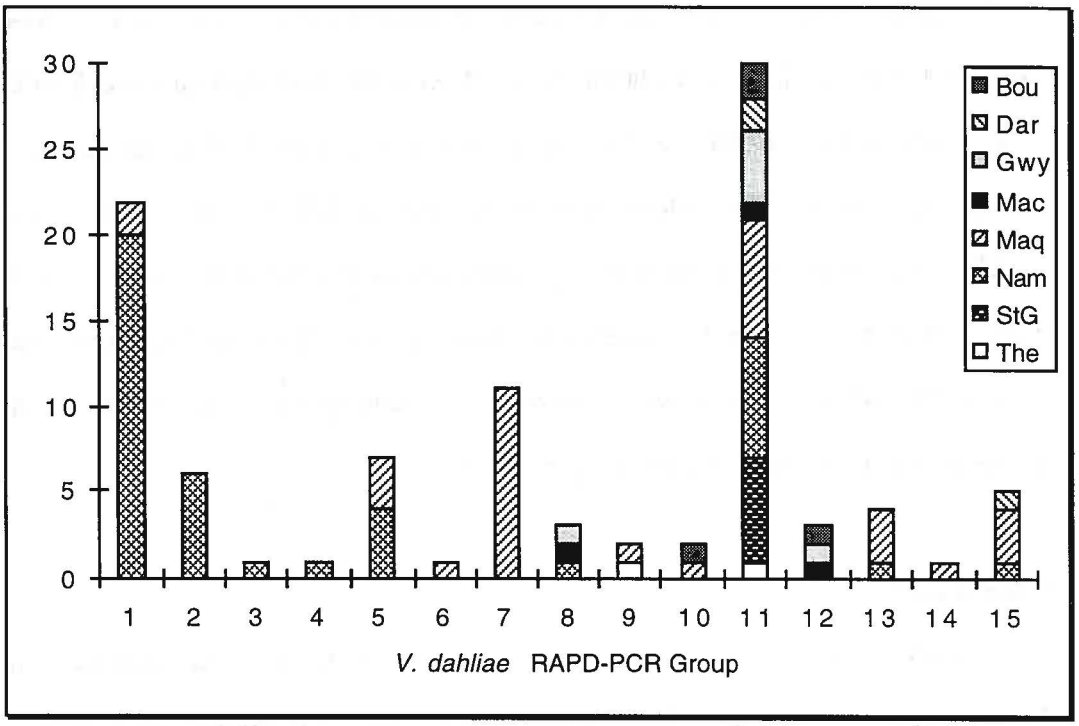


**FIGURE 2.** Chart comparing the numbers of *V. dahliae* isolates from each RAPD-PCR group found in the eight cotton production regions (Bou = Bourke, Dar = Darling Downs, Gwy = Gwydir Valley, Mac = Macintyre Valley, Maq = Macquarie Valley, Nam = Namoi Valley, StG = St George, The = Theodore).

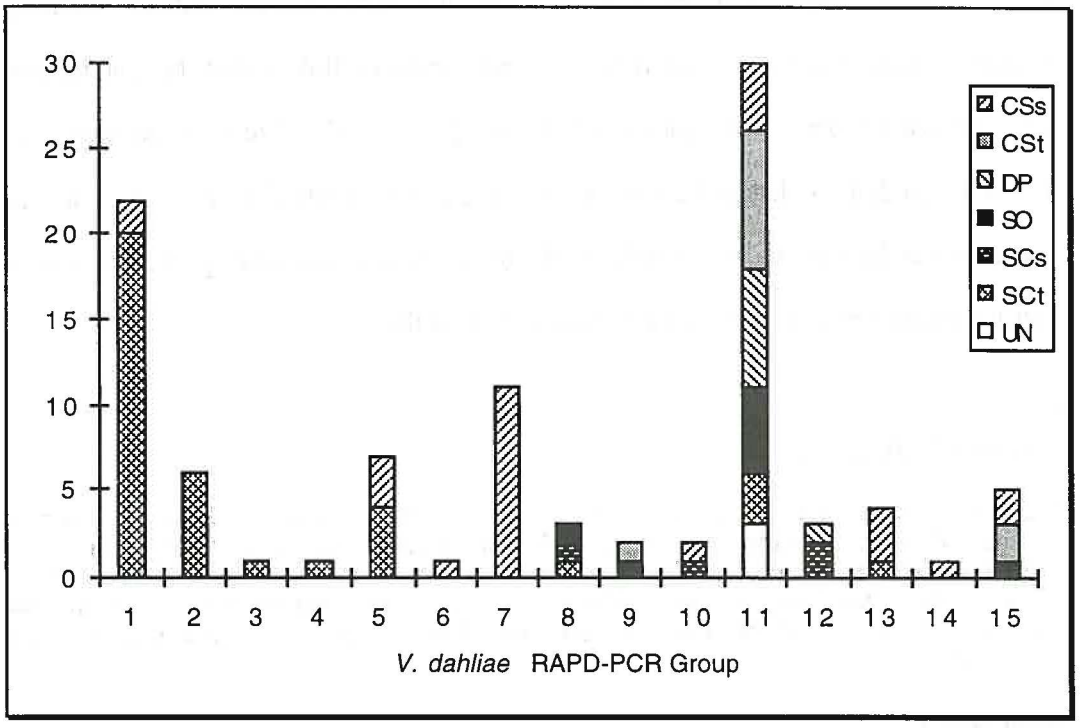


**FIGURE 3.** Chart comparing the numbers of *V. dahliae* isolates from each RAPD-PCR group isolated from different cotton cultivar types (CSs = CS sensitive, CSt = CS tolerant, DP = Deltapine, SO = Siokra, SCs = Sicala sensitive, SCt = Sicala tolerant, UN = unknown).

and Deltapine, as well as from the more disease-tolerant cultivars such as CS 189+, Sicala V-1 and Sicala V-2. Relationships between the host cultivars and RAPD-PCR groups of the *V. dahliae* isolates are presented in Figure 3. Both sensitive and tolerant cultivars were found to be hosts to members of RGs 1, 5, 8, 9, 11, 13 and 15. Moreover, members of the most regionally diverse RAPD-PCR group (RG 11) showed an extremely broad preference for host cultivar. These findings imply that there is little apparent relationship between the genetic type of a *V. dahliae* isolate and its potential to cause disease in a particular host.

## Conclusion

Genetic fingerprinting has proved to be an extremely useful technique for the identification of strains of *V. dahliae* isolated from cotton production regions in NSW and Queensland. This survey highlights the significant genetic diversity evident within populations of *V. dahliae* which infect cotton plants, and clearly shows that a variety of organisms are capable of causing disease even in relatively tolerant cotton cultivars. Unfortunately, no obvious link could be established between isolate identity and pathogenicity on a particular host cultivar. Nonetheless, it is believed that the detailed epidemiological and pathological data obtained in this research will be extremely valuable in the development and testing of new cotton cultivars with enhanced tolerance to Verticillium wilt.

## Acknowledgments

We wish to thank Drs. Stephen Allen and Joe Kochman for supplying *V. dahliae* strains and infected cotton plants from NSW and Queensland, respectively. This work was supported by a Project Grant from the Cotton Research and Development Corporation. Zhu Youyong and Wang Yun-Yue were Visiting Scholars from the Department of Plant Protection, Yunnan Agricultural University, Kumming, Yunnan, P. R. China.

## Reference

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